Panel B: Illustrates the nucleic acid sequences (SEQ ID NOS: 34 and 36) of the forward and reverse PCR primers used to amplify CAR D1 (the complement of the reverse primer sequence is shown). Both primers were tailed with restriction sites (bold type) to facilitate cloning into the pET15b expression vector. Amino acid residues (SEQ ID NOS: 35 and 37) encoded by the primers are shown in single letter code.

In the section of the specification entitled Brief Description of the Drawings, please delete the fourth paragraph, lines 16-21, page 10 and lines 1-3, page 11 and replace it with the following revised paragraph:

Panel C: Illustrates the nucleotide sequences (SEQ ID NOS: 38 and 40) and amino acid sequences (SEQ ID NOS: 39 and 42) of the CAR D1-T7A fusion protein generated by ligation of the CAR D1 PCR product (shown in panel B) to the pET15b expression vector (both the PCR product and the pET15b plasmid were digested with *Nco*I and *Xho*I before ligation). The amino acid sequence of the resulting CAR D1-T7A fusion protein is shown in single letter code (SEQ ID NO: 41) on the top line (note that the central amino acid residues of CAR D1, from Ile 3 to Ala 125, are not shown, and are represented by ...). The translation termination signal is indicated by *. Nucleotide sequences of restriction enzyme cleavage sites used to generate CAR D1-peptide fusion proteins are labeled and shown in bold type.

In the section of the specification entitled Brief Description of the Drawings, please delete the sixth paragraph, lines 10-17, page 11 and replace it with the following revised paragraph:

FIG. 3: Illustrates a schematic of the structure of vectors for fusion of a protein amino-terminus to peptide extensions. DNA fragments encoding the T7B peptide or various modified T7B peptides were amplified by PCR using primers that appended an upstream *NcoI* restriction site (SEQ ID NO: 43) and a downstream *NdeI* restriction site (SEQ ID NO: 44) encoding the amino acid sequences as shown in Panel A. The PCR products were then cloned between the *NcoI* and *NdeI* sites of pET15b, as shown in Panel B. In the final ligated products, the 6-His tag (SEQ ID NO: 46) (which is normally present in pET15b) is replaced by the N-terminal peptides (SEQ ID NO: 45).

In the section of the specification entitled Description of the Invention, please delete the first full paragraph, lines 3-13, page 17 and replace it with the following revised paragraph:

A second nucleic acid sequence is provided which encodes a peptide extension having a net negative charge. The second nucleic acid is fused in-frame to the first nucleic acid in an expression vector such that a fusion protein encoded by the first and second nucleic sequences is expressed in the host cell following transformation of the host cell with the expression vector encoding the fusion protein. The peptide extension encoded by the second nucleic acid sequences is positioned at the carboxyl-terminus of the protein or peptide of interest. The peptide T7A (SEQ ID NO: 20) of Table 1 is specifically excluded in connection with this embodiment. In any jurisdiction which does not recognize a one-year grace period for filing a patent application following the public disclosure of an invention, it may also be necessary to exclude peptides T7B (SEQ ID NO: 6) and T7C (SEQ ID NO: 5) of Table 1 in connection with this and related embodiments.

In the section of the specification entitled Description of the Invention, please delete the second paragraph, lines 9-12, page 19 and replace it with the following revised paragraph:

Examples of specific peptide extensions falling within the scope of the present invention include peptides T7C (SEQ ID NO: 5), T7B (SEQ ID NO: 6), T7B1 (SEQ ID NO: 7), T7B2 (SEQ ID NO: 8), T7B3 (SEQ ID NO: 9), T7B5 (SEQ ID NO: 11), T7B6 (SEQ ID NO: 12), T7B7 (SEQ ID NO: 13), T7B8 (SEQ ID NO: 14), T7B9 (SEQ ID NO: 15), T7B10 (SEQ ID NO: 16), T7B11 (SEQ ID NO: 17), T7B12 (SEQ ID NO: 18), T7B13 (SEQ ID NO: 19), T7A1 (SEQ ID NO: 21), T7A2 (SEQ ID NO: 22), T7A3 (SEQ ID NO: 23), T7A4 (SEQ ID NO: 24) and T7A5 (SEQ ID NO: 25), as shown in Table 1.

In the section of the specification entitled Description of the Invention, please delete the first full paragraph, lines 3-7, page 20 and replace it with the following revised paragraph:

The specific amino-terminal peptide extensions exemplified comprise solubility or activity promoting portions of the 57 residue carboxyl-terminal portion of the T7 gene 10B protein, or variants thereof which result in the maintenance of a net charge ranging +2 to -20. Specifically, disclosed peptides include the following peptides which appear in Table 1: peptides N1 (SEQ ID NO: 27), N2 (SEQ ID NO: 28), N3 (SEQ ID NO: 29), N4 (SEQ ID NO: 30), N5 (SEQ ID NO: 31), N6 (SEQ ID NO: 32) and N7 (SEQ ID NO: 33).

In the section of the specification entitled Exemplification, please delete the first paragraph of the sub-section entitled: "II. Expression of the CAR DI-T7A Fusion Protein", lines 15-21, page 25

and lines 1-15, page 26 and replace it with the following revised paragraph:

Expression of the CAR D1-T7A fusion protein (the sequence of the T7A peptide extension is shown in Table 1) (SEQ ID NO: 20) from the pET15b-CAR D1 construct was performed as follows. The pET15b-CAR D1 construct was transformed into *Escherichia coli* strain BL21-DE3 (Novagen, Inc.). Freshly transformed colonies were used to inoculate Luria-Bertani (LB) broth containing 150 mg/L penicillin G (Sigma), and the culture was grown at 37°C until mid-log phase (optical density approximately 0.8 at 600 nm). The culture was then chilled to 18°C and adjusted to 50 μM isopropyl D-thiogalactopyranoside (IPTG; Aldrich-Sigma) to induce protein expression. After incubation for an additional 5-20 hr at 18-20°C, the cells were harvested and analyzed for expression of CAR D1. Cells were lysed by several cycles of rapid freezing and thawing in the presence of lysozyme, followed by sonic disruption with a probe tip sonicator (Heat Systems, Inc.). Lysates were then centrifuged, and the supernatant fraction was transferred to a fresh tube. Protein content in both the soluble (supernatant) and insoluble (pellet) fractions was examined by SDS-PAGE (electrophoresis in polyacrylamide gels in the presence of sodium dodecylsulfate, a strong detergent and protein denaturant). Experimental results demonstrated that, when CAR D1 was fused to the 22 residue T7A peptide extension (SEQ ID No: 20), approximately 50% of the CAR D1 protein was present in the soluble fraction of cell lysates, whereas the remainder of the CAR D1 fusion protein was present in the insoluble pellet fraction (which contained the macroscopic inclusion bodies). In contrast, when the 22 residue peptide extension was eliminated by insertion of a stop codon upstream of the *Xho*I cloning site, the CAR D1 fusion protein was found to be completely aggregated into insoluble inclusion bodies (*See,* Freimuth, *et al.* (1999)).

In the section of the specification entitled Exemplification, please delete the first paragraph of the sub-section entitled: "III. Specificity of the Peptide-Mediated Folding of CAR D1", lines 8-22, page 27 and lines 1-3, page 28 and replace it with the following revised paragraph:

Additional experiments were performed in order to establish whether the mechanism of CAR D1 folding enhancement was specific for the T7A peptide (SEQ ID NO: 20) derived from the bacteriophage T7 gene 10B protein. The bacteriophage T7 gene 10 encodes two proteins, 10A and 10B, which are identical in amino acid sequence for the first 342 amino acid residues. Translation of the 10A protein is continued for three additional codons before terminating after codon 345, whereas a reading frame shift in codon 343 produces the 10B form which continues translation for a total of 56 additional codons before terminating after codon 398. *See,* Condron, *et al.*, J. Bacteriol. **173**: 6998-7003 (1991). The sequence of the carboxyl-terminal 57 amino acid residues of the bacteriophage T7 gene 10B protein (amino acid residues 343-398) is FQSGVMLGVASTVAASPEEASVTSTEETLTPAQEAARTRAANKARKEAELAAATAEQ (SEQ ID NO: 1). The bacteriophage T7 gene 10A and 10B proteins are structural proteins that form the icosahedral phage head. The unique 57 residue carboxyl-terminus of the 10B protein is exposed on the surface of phage heads, but this peptide is not essential for propagation of bacteriophage T7 under laboratory conditions. Indeed, in the bacteriophage T7-based phage display system (see Novagen catalog and Studier, et al. U.S. Patent No. 5,766,905), foreign peptides are substituted for the non-essential 10B C-terminal 57 residue peptide, and thus become displayed on the phage head.

In the section of the specification entitled Exemplification, please delete the third paragraph of the sub-section entitled: "III. Specificity of the Peptide-Mediated Folding of CAR D1", lines 10-20, page 28 and replace it with the following revised paragraph:

To investigate the specificity of the T7A peptide-mediated folding of CAR D1, the effects of bacteriophage T7 and T3 gene 10B-derived, carboxyl-terminal peptide extensions on the folding of CAR D1 were compared. The DNA fragment encoding the 18 amino acid residue T7A peptide (SEQ ID NO: 20) was excised from the pET15b-CAR D1 construct by digestion with restriction endonucleases *Bam*HI and *Blp*I (*see,* FIG. 1, Panel C) and replaced with PCR products encoding either: (*i*) the complete 57 amino acid residue T7 gene 10B terminal peptide (T7C) (SEQ ID NO: 5); (*ii*) a shorter fragment encoding the terminal 40 amino acid residues of the T7 gene 10B terminal peptide (T7B) (SEQ ID NO: 6); or (*iii*) a fragment encoding the terminal 39 amino acid residues of the bacteriophage T3 gene 10B terminal peptide (T3) (SEQ ID NO: 26). These peptide extensions were designated Peptide T7C (SEQ ID NO: 5), Peptide T7B (SEQ ID NO: 6), and Peptide T3 (SEQ ID NO: 26). The amino acid sequences of these peptide extensions are shown in Table 1.

In the section of the specification entitled Exemplification, please delete the first paragraph of the sub-section entitled: "IV. Mechanism of protein folding by T7-derived peptide extensions", lines 6-19, page 30 and replace it with the following revised paragraph:

A. Role of predicted amphipathic α-helices. Both the T7B and T7C peptides were predicted by sequence analysis algorithms (*e.g.,* Chou/Fasman) to contain two long α-helices, both of which have weak amphiphilic character as revealed by helical wheel projections. It is

conceivable that peptide extensions with weak amphiphilic character could function as *cis*-acting chaperones by interacting transiently with hydrophobic regions of the newly translated polypeptide to prevent aggregation. Accordingly, peptide extension mutants were constructed to determine if amphiphilic α-helical character is necessary for the protein folding activity of these peptides. Peptides T7B2 (SEQ ID NO: 8) and T7B3 (SEQ ID NO: 9) incorporate helix-disrupting proline or glycine residues at the start of the predicted carboxyl-terminal helix, whereas Peptide T7B1 (SEQ ID NO: 7) has a deletion that would disrupt the amphiphilic character of the predicted helix. None of these three modified peptide extensions reduced the yield of soluble CAR D1 produced in *E. coli*. Thus, these results demonstrate that the folding activity of the T7B and T7C peptide extensions does not depend on the ability of these peptides to form amphiphilic α-helices.

In the section of the specification entitled Exemplification, please delete the fourth paragraph of the sub-section entitled: "IV. Mechanism of protein folding by T7-derived peptide extensions", lines 16-22, page 32 and lines 1-2, page 33 and replace it with the following revised paragraph:

The ssrA and T7 peptide extensions are similar in that both are carboxyl-terminal modifications of their substrate proteins. Additionally, the T7 peptide contains a sequence motif (AANKAR) (SEQ ID NO: 2) that is similar to the SspB recognition motif in the ssrA peptide, AANDEN (SEQ ID NO: 3); where **N** is the dominant residue recognized by SspB. However, unlike the ssrA tag, which is always fused to truncated nascent polypeptides, the T7 peptides of the invention disclosed herein are fused to complete, full-length proteins or protein domains. Therefore, if SspB and/or ClpX recognize sequence elements in the T7 peptides, then these factors

conceivably might promote folding rather than degradation of intact proteins or protein domains.

In the section of the specification entitled Exemplification, please delete the fifth paragraph of the sub-section entitled: "IV. Mechanism of protein folding by T7-derived peptide extensions", lines 3-10, page 33 and replace it with the following revised paragraph:

Accordingly, in order to determine whether the T7B peptide acts through a mechanism that is dependent upon binding by SspB and/or ClpX, additional mutants were constructed in which critical residues of the putative recognition sites for either SspB (*i.e.,* Peptide T7B11 (SEQ ID NO: 17) and Peptide T7B12 (SEQ ID NO: 18)) or ClpX (*i.e.,* Peptide T7B9 (SEQ ID NO: 15) and Peptide T7B10 (SEQ ID NO: 16)) were altered or deleted. Experimental results demonstrated that the yield of soluble CAR D1 was not reduced by any of these aforementioned mutations, indicating that these *trans*-acting factors do not contribute to the mechanism of T7B-mediated folding of CAR D1.

In the section of the specification entitled Exemplification, please delete the sixth paragraph of the sub-section entitled: "IV. Mechanism of protein folding by T7-derived peptide extensions", lines 11-21, page 33 and lines 1-8, page 34 and replace it with the following revised paragraph:

C. Role of peptide net charge. During analysis of T7 peptide mutants generated for the studies described above, it was observed that the partial folding-activity of peptide T7A (SEQ ID NO: 20) was increased by mutation to peptide T7A1 (SEQ ID NO: 21), and, conversely, that the

full folding-activity of peptide T7B (SEQ ID NO: 6) was reduced by mutation to peptide T7B4 (SEQ ID NO: 10). The T7A1 (SEQ ID NO: 21) mutant was constructed to disrupt the weak amphiphilic character of the peptide, whereas a T7B4 (SEQ ID NO: 10) mutant was constructed to probe the length-dependence of the folding activity. However, as may be ascertained from Table 1, the mutation in Peptide T7A1 (SEQ ID NO: 21) increases the peptide net charge from -3 to -4, whereas the Peptide T7B4 (SEQ ID NO: 10) mutation decreases the peptide net charge from -6 to -2. Based on these results, additional mutants were constructed in order to systematically examine whether there was a correlation between peptide net charge and ability to mediate folding of CAR D1. As demonstrated by the experimental results not shown here, the relative proportion of soluble CAR D1 produced in *E. coli* increased as the net negative charge on Peptide T7A (SEQ ID NO: 20) was increased from -3 to -6 (peptides T7A1 (SEQ ID NO: 21), T7A2 (SEQ ID NO: 22), and T7A3 (SEQ ID NO: 23)). Both Peptides T7A3 (SEQ ID NO: 23) and T7B (SEQ ID NO: 6) were found to produce almost a 100% yield of soluble CAR D1, and both species had a net negative charge of -6. Therefore, the characteristic of the carboxyl-terminal peptide extensions that is critical for their ability to mediate folding of CAR D1 appears to be the size of the net negative charge carried by the peptide extension. Consistent with this conclusion, the T3 peptide (SEQ ID NO: 26) extension, which is unable to fold CAR D1, has a net charge of -2.

In the section of the specification entitled Exemplification, please delete the second paragraph of the sub-section entitled: "V. Applicability of C-terminal extensions to other test proteins", lines 3-17, page 35 and replace it with the following revised paragraph:

In order to distinguish between these two possible mechanisms, the effect of peptide extensions on the folding of other test proteins was examined. In one experiment, the distal domain of the human A33 protein (Heath, et al., Proc. Natl. Acad. Sci. U.S.A. **94**: 469-474 (1997)), the protein that is most similar to CAR D1 as revealed by homology searching using the BLAST-P program (32% identical), was examined. A33 and CAR are both members of the immunoglobulin superfamily and have similar protein and gene organization. See, Chretien, et al., Eur. J. Imunol. **28**: 4094-4104 (1998). A cDNA fragment encoding the A33 distal domain (D1) was amplified by PCR and cloned into the pET15b-T7A construct in the same manner as schematically illustrated in FIG. 1 for CAR D1. When a stop codon was included to prevent fusion to the T7 peptide, the A33 protein was found to be insoluble, as was also found for CAR D1. However, unlike the results obtained with CAR D1, extending the carboxyl-terminus of A33 D1 with the T7B peptide (SEQ ID NO: 6) did not increase A33 D1 solubility. Therefore, the T7B peptide (SEQ ID NO: 6) does not appear to universally promote protein folding in vivo, supporting the conclusion that these peptides do not function by recruiting chaperones to the misfolded protein.

In the section of the specification entitled Exemplification, please delete the third paragraph of the sub-section entitled: "V. Applicability of C-terminal extensions to other test proteins", lines 18-21, page 35 and lines 1-18, page 36 and replace it with the following revised paragraph:

To determine if further increasing the peptide extension net negative charge would enhance folding of A33 D1, the A33 D1 domain was fused to Peptide T7B7 (SEQ ID NO: 13), which has a net charge of - 12 (see, Table 1). Results demonstrated that the A33 D1-T7B7 fusion protein was

distributed approximately equally between the soluble and insoluble fractions of cell lysates. Only a slight further increase in fusion protein solubility resulted when A33 D1 was fused to Peptide T7B8 (SEQ ID NO: 14) (data not shown), which has a net charge of - 16 (see, Table 1). Because the function of A33 is unknown and consequently there is no assay for its biological activity, the A33 D1-T7B7 conformation was characterized by limited proteolysis. Staphylococcal V8 protease digested the T7B7 (SEQ ID NO: 13) peptide extension more readily than the A33 D1 domain itself, as was observed for CAR D1 fusion proteins, generating digestion products which migrated with slightly faster mobility than the intact protein in SDS-PAGE. However, unlike CAR D1, the A33 D1 domain and the T7B7 (SEQ ID NO: 13) peptide extension were equally sensitive to digestion with trypsin. Thus, although the A33 D1-T7B7 fusion protein is soluble, it may have a non-native conformation. This was further supported by the observation that the A33 D1-T7B7 fusion protein resolves into several species with distinct mobilities when electrophoresed under non-denaturing conditions. Together these results suggested that although the carboxyl-terminal peptide extension was able to partially solubilize A33 D1, it may not be able to mediate proper folding of the domain. Concomitant control experiments showed that both peptides T7B7 (SEQ ID NO: 13) and T7B8 (SEQ ID NO: 14) promote folding of CAR D1 into its biologically active conformation (data not shown), indicating that these peptides are compatible with *in vivo* folding of at least some proteins.

In the section of the specification entitled Exemplification, please delete the fourth paragraph of the sub-section entitled: "V. Applicability of C-terminal extensions to other test proteins", lines 19-21, page 36 and lines 1-12, page 37 and replace it with the following revised paragraph:

The analysis was extended to determine if the folding of other proteins could be enhanced *in vivo* by extending the protein C-terminus with the T7B peptide (SEQ ID NO: 6) and more highly charged derivatives (T7B5-T7B8) (SEQ ID NOS: 11, 12, 13, 14, respectively). The E. coli ClpX protein, a ~50 kD chaperone, misfolds and aggregates into inclusion bodies when overexpressed in E. coli using pET vector technology. ClpX, therefore, is an example of how the conditions of protein overexpression can render E. coli unable to properly fold even its own endogenous proteins. As discussed above, this may result from a deficit of one or more chaperones that are required to fold nascent polypeptide chains. Fusion of the ClpX C-terminus to T7B (SEQ ID NO: 6) or to T7B5-T7B8 (SEQ ID NOS: 11-14, respectively) peptides increased the fraction of the protein that was recovered in the soluble fraction of cell lysates. However, in contrast to the results obtained with A33, the C-terminal peptide extensions could be readily cleaved from the ClpX protein by limited proteolysis with both trypsin and V8 protease. Furthermore, after proteolytic removal of the T7B (SEQ ID NO: 6) C-terminal extension, the resulting processed ClpX protein had full biological activity both in terms of ATPase activity and ability to cooperate with the ClpP proteasome in degrading model protein substrates.

In the section of the specification entitled Exemplification, please delete the fifth paragraph of the sub-section entitled: "V. Applicability of C-terminal extensions to other test proteins", lines 13-19, page 37 and replace it with the following revised paragraph:

A group of thirteen yeast proteins which are known to form inclusion bodies when over-expressed in *E. coli* using pET expression vectors were separately fused to the T7B (SEQ ID NO: 6)

peptide extension. Solubility and folding of six of these proteins was rescued to greater than 50%, while another two were rescued to a lesser extent. Solubility and folding of the remaining five proteins was not measurably affected by the T7B peptide (SEQ ID NO: 6) extension (Table 2). Fusion to C-terminal peptide T7B7 (SEQ ID NO: 13) failed to increase the solubility of these five refractory yeast proteins.

In the section of the specification entitled Exemplification, please delete the third paragraph of the sub-section entitled: "VI. Effect of N-terminal extensions on protein folding *in vivo*", lines 17-20, page 39 and lines 1-2, page 40 and replace it with the following revised paragraph:

This model was tested by fusing the CAR D1 N-terminus to amino-terminal peptide extensions, according to the method outlined in Figure 3. Consistent with the above-stated model, CAR D1 was least soluble when fused to the N-terminal peptide extensions N2 (SEQ ID NO: 28) and N3 (SEQ ID NO: 29) (which have neutral or +1 net charges, respectively). By contrast, CAR D1 was mostly soluble when fused to the N-terminal peptides N1 (SEQ ID NO: 27) and N4 (SEQ ID NO: 30), which have net charges of -2 and +2, respectively.

In the section of the specification entitled Exemplification, please delete the fourth paragraph of the sub-section entitled: "VI. Effect of N-terminal extensions on protein folding *in vivo*", lines 3-11, page 40 and replace it with the following revised paragraph:

Results of further testing with other protein substrates were not completely consistent with

this model, however. For example, the solubility of the 50 kD ClpX protein was significantly increased by fusion to the N-terminal peptide extension N2. Because the N2 peptide (SEQ ID NO: 28) has no net charge, it seems unlikely that this peptide could rescue of the folding of ClpX by a mechanism dependent on peptide net charge. Rather, in this case the N-terminal peptide extension may alter the initial folding pathway of the nascent polypeptide, fortuitously avoiding the formation of folding intermediates that may precipitate or be minimally soluble under conditions of chaperone deficit. Alternatively, the N-terminal peptides may recruit chaperones to the nascent polypeptide chain.

In the section of the specification entitled Exemplification, please delete the first paragraph of the sub-section entitled: "VII. Effects of Peptide Extensions on *In Vitro* Renaturation", lines 13-21, page 40 and lines 1-5, page 41 and replace it with the following revised paragraph:

During *in vitro* refolding of denatured proteins, precipitation and aggregation of the protein upon removal of the denaturing agent is a common side reaction. Thus, precipitation and aggregation are problematic side reactions during the folding of proteins both *in vivo* and during refolding *in vitro*. Since carboxyl-terminal peptide extensions which carry a large net negative charge inhibit protein aggregation *in vivo*, possibly by increasing electrostatic charge repulsion between nascent polypeptide chains, experiments were performed to investigate whether such peptide extensions could inhibit protein aggregation during protein refolding reactions *in vitro*. To test this hypothesis, the A33 D1 protein fragment was produced in 2 different forms, with or without a T7B6 peptide (SEQ ID NO: 12) carboxyl-terminal extension. Both forms of the A33 D1 protein

were produced with an amino-terminal 6-histidine tag. When protein expression was induced at 37°C, both A33 D1 and A33 D1-T7B6 proteins misfolded and accumulated in inclusion bodies (note that A33 D1-T7B6 is only partially soluble when induction is carried out at temperatures below 25°C).

In the section of the specification entitled Exemplification, please delete the first paragraph of the sub-section entitled: "VIII. Production of a synthetic T7A peptide", lines 20-21, page 42 and lines 1-4, page 43 and replace it with the following revised paragraph:

A synthetic peptide corresponding in sequence to peptide T7A was produced, as shown:

(acetyl-cysteine)-LEDPAANKARKEAELAAATAEQ (SEQ ID NO: 4).

An amino-terminal cysteine residue was incorporated into the peptide to introduce a reactive sulfhydryl group which could be utilized to couple the peptide to solid supports or carrier proteins.

In the section of the specification entitled Exemplification, please delete Table I, page 45 and replace it in its entirety to include SEQ ID NOS as shown in the following:

Table 1

Peptide Name	Sequence	Net Charge[a]	SEQ ID NO
T7C	*LEDP*FQSGVMLGVASTVAASPEEASVTSTEETLTPAQEAARTRAANKARKEAELAAATAEQ	-6	5
T7B	*LEDP*----------EEASVTSTEETLTPAQEAARTRAANKARKEAELAAATAEQ	-6	6
T7B1	*LEDP*----------EEASVTSTEETLTPAQEAARTRAANKARKEAEL---TAEQ	-6	7
T7B2	*LEDP*----------EEASVTSTEETLTPAQEAARTR**PP**NKARKEAELAAATAEQ	-6	8
T7B3	*LEDP*----------EEASVTSTEETLTPAQEAARTR**GG**NKARKEAELAAATAEQ	-6	9
T7B4	*LEDP*----------TPAQEAARTRAANKARKEAELAAATAEQ	-2	10
T7B5	*LEDP*----------EEASVTSTEETLTPAQEAARTRAANKARKEAEL**EAE**TAEQ	-8	11
T7B6	*LEDP*----------EEASVTSTEETLTPAQEAA**ETE**AANKARKEAEL**EAE**TAEQ	-12	12
T7B7	*LEDP*----------EEASVTSTEETLTPAQEAARTRAANKA**EE**EAEL**EAE**TAEQ	-12	13
T7B8	*LEDP*----------EEASVTSTEETLTPAQEAA**ETE**AANKA**EE**EAEL**EAE**TAEQ	-16	14
T7B9	*LEDP*----------EEASVTSTEETLTPAQEAARTRAANKARKEAELAA-----	-5	15
T7B10	*LEDP*----------EEASVTSTEETLTPAQEAARTRAANKARKEAELAAA----	-5	16
T7B11	*LEDP*----------EEASVTSTEETLTPAQEAARTRAA**A**KARKEAELAAATAEQ	-6	17
T7B12	*LEDP*----------EEASVTSTEETLTPAQEAARTR---KARKEAELAAATAEQ	-6	18
T7B13	*LEDP*----------EEASVTSTEETLTPAQEAARTRAANK---EAELAAATAEQ	-8	19
T7A	*LEDP*----------AANKARKEAELAAATAEQ	-3	20
T7A1	*LEDP*----------**ER**NK**E**RKEAELAAATAEQ	-4	21
T7A2	*LEDP*----------**ER**NK**E**RKEAEL**E**AATAEQ	-5	22
T7A3	*LEDP*----------**ER**NK**E**RKEAEL**EAE**TAEQ	-6	23
T7A4	*LEDP*----------AANKARKEAEL**E**AATAEQ	-4	24
T7A5	*LEDP*----------AANKARKEAEL**EAE**TAEQ	-6	25
T3	*LEDP*----------AVWEAGKVVAKGVGTADITATTSNGLIASCKVIVNAATS	-2	26
N1	M-EEASVTSTEETLTPAQEAARTRAANKARKEAELAAATAEH	-2	27
N2	MAE**R**ASVTSTEETLTPAQEAARTRAANKARKEAELAAATAEH	0	28
N3	MAE**EAK**VTSTEETLTPAQEAARTRAANKARKEAELAAATAEH	+1	29
N4	MAE**RAKR**TSTEETLTPAQEAARTRAANKARKEAELAAATAEH	+2	30
N5	M-EEASVTSTEETLTPAQEAARTRAANKARKEAEL**EAE**TAEH	-4	31
N6	M-EEASVTSTEETLTPAQEAA**ETE**AANKARKEAEL**EAE**TAEH	-8	32
N7	M-EEASVTSTEETLTPAQEAARTRAANKA**EE**EAEL**EAE**TAEH	-8	33

[a]The terminal COO^- and NH_3^+ groups of carboxyl-terminal and amino terminal peptide extensions were included in the calculation of peptide net charge